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Serological survey of *Toxoplasma gondii*, *Dirofilaria immitis*, Feline Immunodeficiency Virus (FIV) and Feline Leukemia Virus (FeLV) infections in pet cats in Bangkok and vicinities, Thailand

Woraporn Sukhumavasi[a,*], Mary L. Bellosa[b], Araceli Lucio-Forster[b], Janice L. Liotta[b], Alice C.Y. Lee[b], Pitcha Pornmingmas[c], Sudchit Chungpivat[a], Hussni O. Mohammed[d], Leif Lorentzen[e], J.P. Dubey[f], Dwight D. Bowman[b]

[a] *Parasitology Unit, Department of Pathology, Faculty of Veterinary Science, Chulalongkorn University, Henri-Dunant Rd., Bangkok 10330, Thailand*
[b] *Department of Microbiology and Immunology, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853, USA*
[c] *Suvarnachad Animal Hospital, 33/39 Moo 3, Ramkamhang Rd., Sapansoong, Bangkok 10240, Thailand*
[d] *Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853, USA*
[e] *IDEXX Laboratories, Westbrook, ME 04092, USA*
[f] *U.S. Department of Agriculture, Animal Natural Resources Institute, Animal Parasitic Disease Laboratory, BARC-East, Building 1001, Beltsville, MD 20705-2350, USA*

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ABSTRACT

The seroprevalence of *Toxoplasma gondii*, *Dirofilaria immitis* (heartworm), feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV) infections was examined using serum or plasma samples from 746 pet cats collected between May and July 2009 from clinics and hospitals located in and around Bangkok, Thailand. The samples were tested for heartworm, FIV, and FeLV using a commercial ELISA. Of the 746 samples, 4.6% (34/746) were positive for heartworm antigen, 24.5% (183/746) had circulating FeLV antigen, and 20.1% (150/746) had antibodies against FIV. In addition, the first 348 submitted samples were tested for *T. gondii* antibodies using a modified agglutination test (MAT, cut off 1:25); 10.1% (35/348) were seropositive. Of the 348 cats sampled for all four pathogens, 11, 10, and 1 were positive for *T. gondii* antibodies and FIV antibodies, FeLV antigen, or *D. immitis* antigen, respectively. Of the 35 *T. gondii*-seropositive cats, 42.9% (15/35) were co-infected with at least one of the other three pathogens. The presence of antibodies to FIV was significantly associated with both age and gender, while FeLV antigen presence was only associated with age. In the case of FIV, males were twice as likely to be infected as females, and cats over 10 years of age were 13.5 times more likely to be infected than cats less than 1 year of age. FeLV antigen was more common in younger cats, with cats over 10 years of age being 10 times less likely to be FeLV positive than cats under 1 year of age. This is the first survey for these four pathogens affecting feline health in Thailand.

1. Introduction

Heartworm (*Dirofilaria immitis*), *Toxoplasma gondii*, feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV) are important infectious agents in cats. Cats can succumb to infection by *D. immitis* with clinical signs varying from asymptomatic to chronic respiratory

* Corresponding author. Tel.: +662 218 9663 70/668 5 351 1080; fax: +662 218 9666.
E-mail address: vetkwan@hotmail.com (W. Sukhumavasi).

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signs, and acute death (Litster and Atwell, 2008; Bowman and Atkins, 2009). Also, pulmonary larval dirofilariasis or heartworm-associated respiratory disease (HARD) is associated with asthma-like clinical signs in cats (Venco et al., 2008). *T. gondii* is a zoonotic agent that infects people and other animals worldwide (Dubey and Beattie, 1988; Dubey, 2010); cats serve as a definitive host and are the only source of the oocysts in the environment. *T. gondii* infections in cats are typically asymptomatic, but the infection in other mammals and birds can cause severe disease with the most feared consequence being the infection of a human fetus during pregnancy in a naïve mother (Elmore et al., 2010). FIV and FeLV are retroviral disease agents of cats worldwide (Dunham and Graham, 2008). Prior studies of cat sera have shown an association between seropositivity for *T. gondii* and these two viral agents in several countries (Witt et al., 1989; Lin et al., 1990, 1992; Dorny et al., 2002; Maruyama et al., 2003; Dubey et al., 2009). Here, we investigated the prevalence of these four agents in pet cats in Thailand and examined the potential association of infection with the pathogens in the same host and the possible risk factors that might be associated with infection with these agents.

2. Materials and methods

2.1. Animals and samples

Serum or plasma samples were obtained from 746 pet cats between May and July 2009 from 137 clinics and hospitals located in and around Bangkok. Samples were brought to the laboratory for this testing if sufficient sera or plasma remained after other in-clinic diagnostics were performed. Samples were stored at −80 °C, thawed, and vortexed prior to serological testing. Only one sample was collected per cat. Signalment and history information, i.e., gender, age, habitat (totally indoors or access to the outdoors), was collected retrospectively from each clinic and hospital.

Gender was recorded for 477 (63.9%) of the 746 cats (Table 1). Age was recorded for only 292 (39.1%) of the 746 cats. Whether a cat was indoors only or had access to the outdoors was recorded for 133 (17.8%) of the 746 cats, and 39.8% of these cats were considered indoor cats. In the case of the subset of 348 cats sampled for antibodies to *T. gondii*, gender was recorded for only 225 cats (64.7%), age for 148 cats (42.5%), and indoor–outdoor status for 77 cats (22.1%) of which 40.3% were considered indoor cats.

2.2. Detection of heartworm, FIV and FeLV

The assay utilized to detect the circulating antigens of *D. immitis* and FeLV and specific antibodies to FIV was the SNAP® Feline Triple® Test (IDEXX Laboratories, Westbrook, ME, USA). The serum or plasma in this case was an aliquot of each sample shipped on dry ice to the United States. The assays were performed as per the manufacturer's instructions.

2.3. Modified agglutination test (MAT)

It was only possible to perform the MAT on about one-half of the samples. Thus, the first 348 submitted samples

Table 1
Age and gender distribution of cats sampled (n = 746) in Bangkok and its surroundings in Thailand for antigens of *D. immitis* and FeLV, FIV antibody, and the subset of cats sampled for antibodies to *T. gondii* (n = 348). Number of male and female cats in Bangkok, Thailand that were sampled and the numbers positive for *D. immitis*, FeLV, FIV (n = 274), and *T. gondii* (n = 140) for which both age and gender had been recorded.

Age (years)	Total 746 sampled cats					Subset of 746 cats of known age and gender sampled for *D. immitis*, FeLV, and FIV									Total 348 sampled cats					Subset of 348 cats of known age and gender sampled for *T. gondii*		
						D. immitis positive			FeLV positive			FIV positive								*T. gondii* positive		
	M	F	TR[a]	GNR[c]	Total	M	F	Total	M	F	Total	M	F	Total	M	F	TR[a]	GNR[c]	Total	M	F	Total
<1	12	11	23	0	23	1	1	2	4	6	10	0	1	1	8	6	14	0	14	0	0	0
1–5	90	65	155	7	162	6	1	7	33	25	58	30	11	41	44	25	69	2	71	5	4	9
6–10	46	33	79	7	86	7	1	8	9	5	14	19	9	28	27	20	47	4	51	2	2	4
>10	7	10	17	4	21	0	1	1	1	0	1	3	3	6	5	5	10	2	12	0	1	1
TR[a]	155	119	274	18	292	14	4	18	47	36	83	52	24	76	84	56	140	8	148	7	7	14
ANR[b]	93	110	203	251	454										44	41	85	115	200			
Total	248	229	477	269	746										128	97	225	123	348			

M: male;
F: female.
[a] TR, Totals where gender and/or age were recorded.
[b] ANR, age not recorded.
[c] GNR, gender not recorded.

Table 2
Prevalence of *T. gondii* (*n* = 348), FIV, FeLV, and/or *D. immitis* (*n* = 746) in cats in Thailand.

Agent(s)	Number positive	Percentage positive	Total positive for any listed agent	Percentage co-infected out of cats with any listed agent
D. immitis	34	4.6%	34	N.A.
FeLV	183	24.5%	183	N.A.
FIV	150	20.1%	150	N.A.
T. gondii	35	10.1%	35	N.A.
FIV and FeLV	41	5.5%	333	12.3%
FIV and *D. immitis*	10	1.3%	184	5.4%
FeLV and *D. immitis*	6	0.8%	217	2.8%
FIV, FeLV, and *D. immitis*	1	0.1%	351	0.3%
T. gondii and FIV	11	3.2%	109	10.1%
T. gondii and FeLV	10	2.9%	118	8.5%
T. gondii and *D. immitis*	1	0.3%	46	2.2%
T. gondii, FIV, and FeLV	6	1.7%	171	3.5%
T. gondii, FeLV, and *D. immitis*	1	0.3%	118	0.9%
T. gondii, FIV, and *D. immitis*	1	0.3%	108	0.9%
T. gondii, FIV, FeLV, *D. immitis*	1	0.3%	161	0.6%

N.A.; not applicable.

were examined using the MAT to detect IgG antibody specific for *T. gondii*. The assay was performed as previously described (Dubey and Desmonts, 1987). A titer of 1:25 was considered positive.

2.4. Data analyses

Descriptive statistics on the percentage of single infections, concurrent infections, gender, and different age groups was performed. The significance of potential association between infection and age, gender, or indoor–outdoor status of the cats, was evaluated using the Chi-square test and was evaluated at $p < 0.05$. This same test was used to examine whether there was any association between all pairwise comparisons with the different agents. In the case where *T. gondii* was used, only the 348 cases that were tested for antibodies to *T. gondii* were included. For the statistical comparisons between antigen or antibody status and gender, age, and indoor–outdoor status, comparisons were only made for subsets where all variables (age, gender, and indoor–outdoor status) had been recorded.

The relation between the age of the cat and the likelihood of seroconversion to FIV and FeLV was evaluated using logistic regression analysis. The age of the cat was divided into 4 categories (<1 year of age, 1–5 years old, 6–10 years old, and >10 years of age). The significance of association of these categories to the reference category (<1 year of age) was evaluated by the significance of the respective regression coefficients and the magnitude of the relation was quantified using the odds ratio (OR) and the 95% confidence interval. The significance was considered at $p < 0.05$. All data analyses were carried out using the Minitab 16.1.1 statistical software (Minitab Inc., State College, PA, USA).

3. Results

Antigens or antibodies to *D. immitis*, FeLV, FIV were found in 4.6%, 24.5%, and 20.1% of 746 cats, respectively; infection with both FIV and FeLV was the most common co-infection (12.3% of 333 cats positive for either pathogen) (Table 2). *T. gondii* antibodies were detected in 10.1% of 348 cats tested. Overall, for the 348 cats tested for the 4 different agents, there were 126 positive for a single agent, 29 positive for two agents, 5 positive for three agents, and a single cat positive for all four agents. This single cat positive for all four agents was a male of unknown age and indoor–outdoor status (Table 2). Of the 35 cats that were positive for *T. gondii*, 15 (42.9%) were also positive for one or more of the other agents for which testing was performed: 1 (2.9%) had antigens of heartworm; 10 (28.6%) had antigens of FeLV; and 11 (31.4%) had antibodies to FIV.

Overall, for the 35 cats with *T. gondii* antibodies, 13 were male and 8 were female (gender was not recorded for 14 cats) (Table 1). Relative to age, there were 9 cats between 1 and 5 years of age (5 males and 4 females), 4 cats from 6 to 10 years of age (2 males and 2 females), and 1 female cat that was over 10 years of age. For the cats of unknown age, 6 were males, 1 was female, and for 14, gender had not been recorded.

Only two factors were significantly associated with the likelihood of seroconversion to FIV; the indoor–outdoor status of the cats did not prove to be significant. Male cats were twice as likely to have seroconverted to FIV (Odds Ratio OR = 2, 95% CI 1.2–3.6). With regard to age, the likelihood of seroconversion increased with the age of the animal; cats above 6 years of age were 12 times more likely than <1 year-old cats to have seroconverted (Odds Ratios: 1–5 years = 7.8; 6–10 years = 12.0; >10 years = 13.5).

In the case of FeLV, only age was associated with a likelihood of antigen being present in the blood of the cats. The likelihood of antigen being present decreased with age, where older cats were 10 times less likely (OR = 0.1) to have circulating antigen in their blood than the younger cats.

4. Discussion

In the present study 1 in 4 cats was positive for FeLV antigen, 1 in 5 for antibodies to FIV, 1 in 10 for antibodies to *T. gondii*, and about 1 in 20 cats had heartworm

Table 3
Comparison of prevalence data from surrounding regions in Southeast Asia; no reports were found for Myanmar, Cambodia, or Laos.

Country	Number of cats examined	Method of testing[a]	Positive for *D. immitis* (%)	Positive for FeLV (%)	Positive for FIV (%)	Positive for *T. gondii* (%)	Reference
Thailand	746	ELISA, MAT	4.6%	24.5%	20.1%	10.1%[b]	Current paper
Thailand	115	RIM	6.1%	–	–	–	Litster and Nilkumhang (2003a)
Thailand	52	PCR	1.9%	–	–	–	Nuchprayoon et al. (2006)
Thailand	315	SFDT	–	–	–	7.3%	Sukthana et al. (2003)
Thailand	592	LAT	–	–	–	11.0%	Jittapalapong et al. (2007)
Thailand	1,490	SFDT	–	–	–	4.8%	Jittapalapong et al. (2010)
Thailand	145	IgG ELISA	–	–	40%	–	Nilkumhang et al. (1994)
Thailand	28	IgG ELISA	–	–	32.1%	–	Pusoonthornthum et al. (1998)
Thailand	653 (stray cats) 110 (sick cats)	Ag ELISA	– –	0.9% 20.9%	–	–	Nilkumhang et al. (1988)
Thailand	115	Ag ELISA (FeLV), IgG ELISA (FIV)	–	6%	5%	–	Litster and Nilkumhang (2003b)
Thailand	133	Ag ELISA (FeLV), IgG ELISA (FIV)	–	14.3%	12.8%	–	Sattasathuchana et al. (2009)
China	278	CAg & IgG ELISA	–	–	–	4.7%	Xie et al. (2010)
China	114	IgG ELISA	–	–	–	23.7%	Chen et al. (2005)
China	206	IgG ELISA	–	–	–	25.2%	Zhang et al. (2009)
Malaysia	101	Ag ELISA	–	3.0%	–	–	Noor and Cheng (1983)
Malaysia	200	Oocysts	–	–	–	13%	Shanta et al. (1980)
Malaysia	477	Mf ID	0.2%	–	–		Mak et al. (1980)
Malaysia	100	Necropsy	3%	–	–	–	Amin-Badjee (1978)
Malaysia	55	IFAT	–	–	–	14.5%	Chandrawathani et al. (2008)
Vietnam	50	Ag ELISA (FeLV), IgG ELISA (FIV), RIM (FeLV Ag, FIV Ab), IFAT (FIV)	–	0%	22%	–	Nakamura et al. (2000)
Vietnam	69	Ag ELISA (FeLV), IgG ELISA (FIV), RIM (FeLV Ag, FIV Ab), IFAT (FIV)	–	0%	0%	–	Miyazawa et al. (1998)
Vietnam	155	LAT	–	–	–	72.3%	Hosono et al. (2009)
Philippines	1	Necropsy	Present	–	–	–	Manuel and Peneyra (1967)
Philippines	125	Ag ELISA	–	11.2%	–	–	Carlos et al. (1992)
Singapore	722	LAT and Toxocell AD	–	–	–	30.7%	Chong et al. (1993)
Indonesia	69	IHA	–	–	–	59.4%	Durfee et al. (1976)

[a] ELISA, enzyme-linked immunosorbent assay; MAT, Modified Agglutination Test; RIM, Rapid Immuno-Migration Assay; PCR, Polymerase Chain Reaction; SFDT, Sabin-Feldman Dye Test; CAg, Circulating Antigen; Mf ID, Microfilarial Identification; LAT, Latex Agglutination Test; IFAT, Indirect Fluorescence Antibody Test; Biokit S.A., Barcelona, Spain.
[b] 348 cats tested (subset of 746 cats).

burdens of sufficient magnitude to be detectable on an antigen test. Seroprevalence of antibodies to *T. gondii* varies among countries, within different areas of a country, and within the same city (Dubey, 2010). The reasons for these variations are many, and generalizations cannot reliably be made. For example, it has been suggested that the low seroprevalence (7.3–11%) in Bangkok, Thailand can be attributed to the lifestyle of the surveyed cats (Sukthana et al., 2003). The majority (95%) of the population of Thailand is Buddhist, and killing any animal is sinful. There are approximately 15,000 cats (30 × 500 temples) that live permanently around Buddhist temples, and these cats are fed mainly cooked fish and rice by the public or monks, possibly reducing feeding on rodent or avian prey.

Although there have been surveys on the prevalence of *T. gondii* in cats in Southeast Asia (Table 3), the present study is the first comprehensive survey for four pathogens. Surveys for FeLV and FIV feline infections in Thailand

have been published in local journals or proceedings and are not available for wider readership (Nilkumhang et al., 1988, 1994; Pusoonthornthum et al., 1998; Litster and Nilkumhang, 2003b; Sattasathuchana et al., 2009). The 10% *T. gondii* seroprevalence in our study is similar to prior reports from Thailand, lower than that reported for Malaysia, Vietnam, and surrounding areas of China (Table 3), and lower than the 25% prevalence reported for cats worldwide (Dubey, 2010).

In the case of heartworm, it would appear that 4.6% of cats tested have sufficient worm burdens, i.e., more than one female heartworm, to allow detection by the antigen assay that was used. This is in agreement with a prior study in Malaysia where 3% of 100 cats were positive at necropsy (Amin-Badjee, 1978). Since heartworms do not routinely develop patent infections with circulating microfilariae in cats, it is not surprising that only 0.2% of cats in Malaysia were positive when blood was examined for the presence of microfilariae (Mak et al., 1980). There does not appear to be any cross-reaction in the SNAP® Feline Triple® Test between *D. immitis* and the other common canine *Dirofilaria* species, *D. repens* (Pantchev et al., 2009).

FeLV was highly prevalent in this population of cats with nearly 25% of the cats being antigen positive. There is unfortunately little information on the extent of disease caused by FeLV in cats in Thailand where vaccination is not routine. Surprisingly, prevalence seems to have increased in the past decade (Litster and Nilkumhang, 2003b; Sattasathuchana et al., 2009). The significant lack of FeLV-positive cats over 5 years of age may be due to the cats, possibly young and susceptible to FeLV infection, succumbing to an FeLV-related disease within 3 years after becoming persistently viremic and not living as long as uninfected cats. Alternatively, older cats may have been capable of developing protective immunity or becoming latently infected hence not being antigenemic or viremic (Dunham and Graham, 2008). The prevalence of FeLV in cats in Thailand was much higher than reported elsewhere in Southeast Asia especially Vietnam which had a total lack of FeLV in 119 cats sampled (Table 3).

The number of cats exposed to FIV was also very high in Thailand. In the present study, as in other studies worldwide, there were more male cats infected than expected by chance alone, which can be explained by the spread of the virus between cats by fighting.

FeLV-positive cats less than 1 year of age were found more commonly than FIV-seropositive cats of the same age. This may be due to the differences in age and behavior of cats prone to be infected by either pathogen. Male adult cats and free-roaming cats are most likely to be infected by biting which is a major route of FIV transmission. In contrast, FeLV transmission mostly occurs via close contact among friendly cats. Also, young kittens are particularly more susceptible to FeLV (Hosie et al., 2009; Lutz et al., 2009). Akhtardanesh et al. (2010) reported seroprevalences of 32.1% for *T. gondii*, 19.2% for FIV, and 14.2% for FeLV in 140 cats from Iran and seropositivity for all 3 infections was higher in older cats. In general FIV and FeLV infection rates are higher in stray male cats. There is little information on co-infections in cats in Asia.

Conflict of interest

The authors have no conflict of interest.

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